"Bioaccumulation of hydroxylated polychlorinated biphenyls and pentachlorophenol in the serum of northern elephant seal pups (Mirounga angustirostris)"

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Abstract

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Bioaccumulation of hydroxylated polychlorinated biphenyls and pentachlorophenol in the serum of northern elephant seal pups (*Mirounga angustirostris*)

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ABSTRACT

Northern elephant seals (NES) (*Mirounga angustirostris*) from the Año Nuevo State Reserve (CA, USA) were sampled at 1-, 4-, 7- and 10-week post-weaning. Concentrations of hydroxylated polychlorinated biphenyls (HO-PCBs) and their parent PCBs were measured in the serum of each individual. The ΣHO-PCB concentrations in the serum increased significantly between early and late fast (from 282 ± 20 to 529 ± 31 pg/mL). This increase might result from a mobilisation of HO-PCBs transferred from the mother during gestation and/or lactation and stored in the pup's liver. Food deprivation has been shown to exacerbate biotransformation capacities in mammals, birds and fish. The HO-penta-CBs was the predominant homologue group, followed by HO-hexa-CBs and HO-hepta-CBs. No preferential pathway for the metabolism of HO-PCBs (HO-direct insertion or NIH-shift of a chlorine atom) could be evidenced. The concentrations of pentachlorophenol (PCP) in the serum of weaned NES increased from 103 ± 7 pg/mL at early fast to 246 ± 41 pg/mL at late fast, which is within the range of PCP concentrations usually encountered in marine mammals.

1. Introduction

Polychlorinated biphenyls (PCBs) are widely spread in the marine and terrestrial biota and biomagnify throughout food chains (Covaci et al., 2002; Letcher et al., 2010). Belonging to a high trophic level, northern elephant seals (NES) (*Mirounga angustirostris*) face an important risk for exposure to environmental lipophilic pollutants. During a 25-day period, NES pups are fed with maternal milk, which contains large amounts of fat and lipophilic pollutants, such as PCBs (Debier et al., 2012). Once absorbed by the animals, PCBs tend to accumulate in lipid-rich tissues and mainly in the adipose tissue (Debier et al., 2006; Louis et al., 2014). During the post-weaning fast, pups mobilise primarily lipids from their large subcutaneous adipose stores in order to meet the needs of animals undergoing a period of development and to prevent protein catabolism (Noren et al., 2003). They thus undergo both fast and development, simultaneously. Although adipose tissue is considered as an internal site of storage for PCBs (La Merrill et al., 2013), they appear to be mobilised from adipocytes into the bloodstream during this period of negative energy balance (Debier et al., 2006; Louis et al., 2014). A release of such toxic pollutants in the blood stream may be problematic since they may reach target tissues and exert harmful health effects in animals (Vanden Berghe et al., 2013).

Seals appear to be capable of biotransforming PCBs, to some extent, through the phase I and II biotransformation process, resulting in the formation of more polar metabolites, such as HO-PCBs, $(HO)_2$-PCBs and methylsulfonyl-PCBs ($MeSO_2$-PCBs) (Nyman et al., 2001; Routti et al., 2008; Teramitsu et al., 2000). Several studies demonstrated the presence of different forms of cytochrome P450 (CYP) (e.g. CYP1A, CYP3A), the phase I enzymes, in ringed (*Phoca hispida*), harbour (*Phoca vitulina*), harp (*Phoca groenlandica*) and grey (*Halichoerus grypus*) seals (Nyman et al., 2000; Ruus et al., 2002; Tilley et al., 2002; Wolkers et al., 1998, 2000). Phase II enzymes, such as UDP glucuronosyl transferase (UDPGT) and glutathione S-transferase (GST), were shown in ringed and harbour seals (Ruus et al., 2002; Wolkers et al., 1998).

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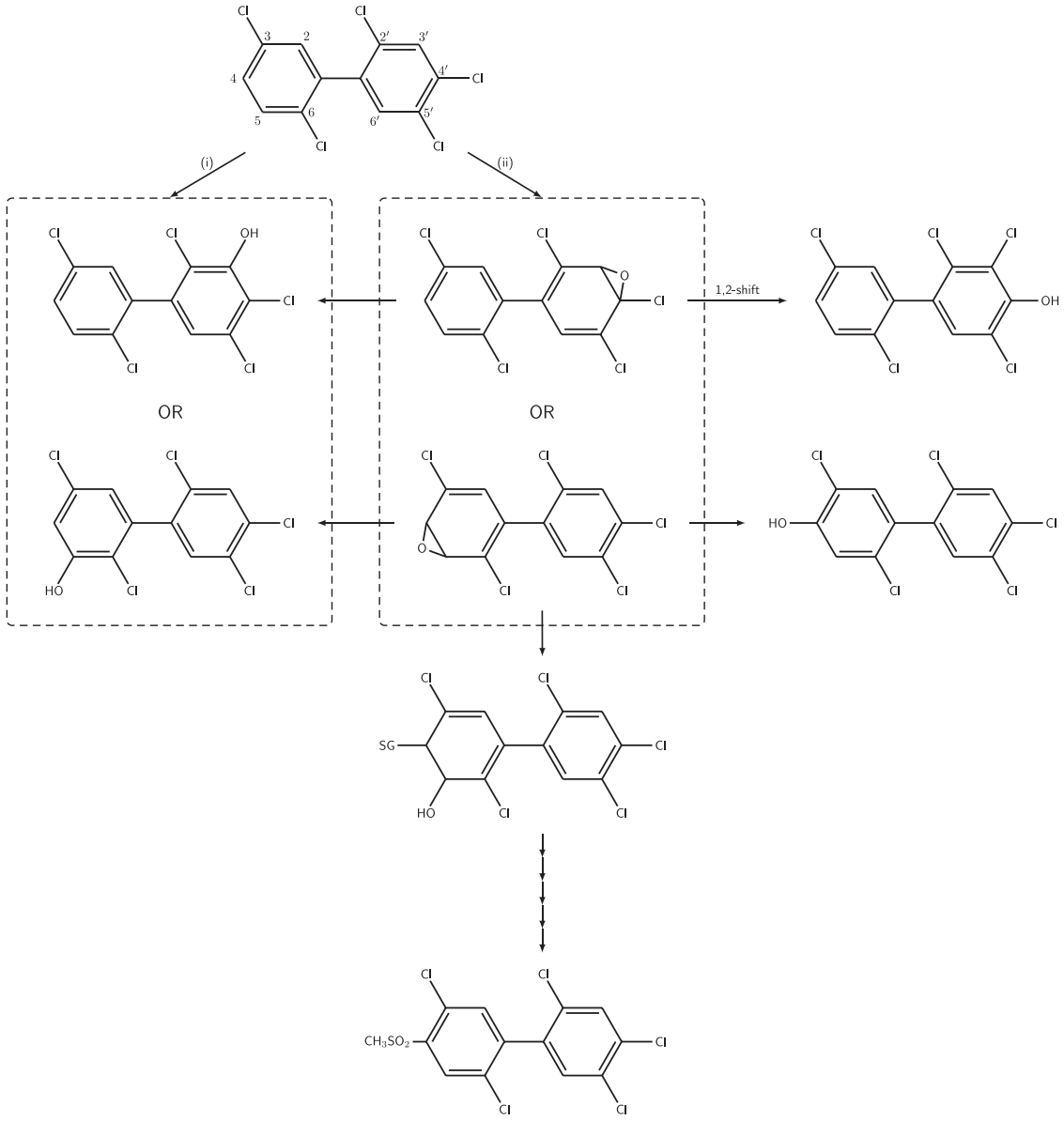

Fig. 1. Metabolism scheme of PCBs. PCB-101 has been chosen as an example. The formations of HO-PCBs by (i) direct HO-insertion and (ii) by NIH-shift, and $MeSO_2$-PCBs are shown. The positions 2, 2′, 6 and 6′ are termed *ortho*. The positions 3, 3′, 5 and 5′ are termed meta and the positions 4 and 4′, *para* (adapted from Letcher et al. (2000)).

The HO-PCBs, resulting from phase I metabolism, may be obtained by (i) the direct insertion of an HO-group, or (ii) the formation of a *meta-para*-epoxide (or arene oxide) intermediate that results in a *meta*- or *para*-HO-PCB metabolite. The *meta-para*-epoxide intermediate with a *meta*-hydrogen and a *para*-chlorine can result in both meta- and *para*-HO-PCB metabolites, the *para*-HO-PCB metabolite being a consequence of 1,2-shift (NIH-shift) (Fig. 1) (Daly et al., 1972; Letcher et al., 2000). The arene oxide may also conjugate with glutathione (GSH) via glutathione-S-transferase (phase II reaction; Zamek-Gliszczynski et al. (2006)). $MeSO_2$-PCBs are then formed further via cascade reactions (details not shown). HO-PCBs being able to bind to specific proteins, they have been detected in the blood compartment of seals (Vanden Berghe et al., 2012). They have also been detected in seal liver (Park et al., 2009). $MeSO_2$-PCBs have been mainly found in the liver, followed by blubber and lung (Larsson et al., 2004).

Biotransformation of PCBs is an important step required for their excretion. However, in numerous cases, it appears that metabolites, and especially HO-PCBs, can exert negative effects on health. Among others, they can lead to endocrine disruptions while competing with estrogens and thyroxin for the corresponding nuclear receptors (Connor et al., 1997; Lans et al., 1993; Van den Berg et al., 1991). They also affect the thyroxin (T_4) metabolism by inhibiting its sulfation (Schuur et al., 1998). HO-PCBs have been shown to disrupt the blood transport of T4 and retinol (Van der Plas et al., 2001). They inhibit mitochondrial oxidative phosphorylation (Narasimhan et al., 1991). *In vitro* studies have also shown that some HO-PCBs exert neurotoxic effects during development leading to locomotor defects, hearing loss, and learning and memory disorders (Meerts et al., 2004).

Marine mammals are also vulnerable to other organic pollutants such as pentachlorophenol (PCP) (Dupont et al., 2013; Routti et al., 2009; Sandau et al., 2000b), a metabolite of hexachlorobenzene (HCB) (Renner, 1988). Similar to HO-PCBs, PCP binds to plasma proteins (Uhl et al., 1986) and has the capability of interacting with thyroid hormone blood transport (Van den Berg et al., 1991). Indeed, its affinity for thyroid hormone transport protein transthyretin (TTR) is twice as high as T_4. PCP is also known to affect the metabolism of thyroid hormone, which could

lead to neurodevelopmental effects in newborns (Sandau et al., 2002).

The aim of the present work was to investigate the levels of HO-PCBs, which appear to be the most important metabolites present in the serum of seals (Vanden Berghe et al., 2012). Several studies have investigated HO-PCBs in young marine mammals (Bytingsvik et al., 2012; Gabrielsen et al., 2011; Villanger et al., 2013; Weijs et al., 2009) including a study by our research group (Vanden Berghe et al., 2012), but never in NES. We compare the changes of HO-PCB contents with their potential PCB precursors in order to investigate trends in PCB metabolism pathways. The levels of PCP and HCB in the serum of NES pups were also evaluated.

2. Material and methods

2.1. NES sampling

Between January and April 2010, twenty-two free-ranging NES pups from Año Nuevo State Reserve (CA, USA) were longitudinally sampled at 1-, 4- and 7-week post-weaning. Only fourteen of the twenty-two NES pups could be recaptured and sampled at 10-week post-weaning. All targeted animals were followed thanks to specific marks of dye during the studied period. NES pups were immobilised with an injection of Telazol at 1 mg/kg of estimated body mass and sedation was then maintained through subsequent intravenous injections of Ketamine (all drugs were from Fort Dodge Animal Health, Fort Dodge, IA, USA) as needed. Blood was collected from the extradural vein into Vacutainer serum tubes (Becton–Dickinson, Erembodegem, Belgium). Samples were centrifuged at 1300x*g* for 15 min at 4 °C and serum was aliquoted into microtubes and stored at −20 °C until analysis (Louis et al., 2014).

2.2. POP analyses

After thawing, serum samples were analysed as reported in Vanden Berghe et al. (2012). Briefly, internal standards (4′-HO-CB159 and CB-143) were added to 1.5 mL serum followed by deionized water and formic acid. The mixture was sonicated for 20 min and loaded on solid-phase-extraction (SPE) cartridges (200 mg/3 mL, Oasis HLB, Waters, Milford, MA). Pollutants were eluted with dichloromethane/methanol (1:1, *v:v*). Extracts were then dried and reconstituted in hexane. A fractionation step on silica SPE cartridges (500 mg/3 mL, VWR, Belgium) topped with acidified silicagel (22% H_2SO_4, *w:w*) was necessary to separate neutral compounds (PCBs and HCB) from phenolic compounds (HO-PCBs and PCP). Neutral compounds were eluted first with 6 mL *n*-hexane, evaporated and reconstituted in iso-octane, while phenolic compounds were eluted with 8 mL dichloromethane, evaporated and derivatized with trimethylsilyl-diazomethane for 30 min at 60 °C. After solvent evaporation, residues were reconstituted in iso-octane. Twelve HO-PCBs (4-HO-CB107, 4-HO-CB120, 4′-HO-CB127, 4′-HO-CB130, 3-HO-CB138, 4-HO-CB146, 4-HO-CB162, 4-HO-CB163, 4′-HO-CB172, 4-HO-CB187, 4-HO-CB193 and 4-HO-CB208; Maervoet et al. (2004)) were analysed as well as 30 PCB congeners (IUPAC numbers: CB-28, -47, -49, -52, -74, -95, -99, -101, -105, -110, -118 , -128, -138, -146, -149, -151, -153, -156, -170, -171, -172, -174, -177, -180, -183, -187, -194, -199, -206 and -209).

All compounds were measured with an Agilent 6890 gas chromatograph coupled with a 5973 mass spectrometer system (GC–MS). All analytical details as well as performed quality assurance and quality control are available in Vanden Berghe et al. (2012).

2.3. Statistical analysis

Data were log transformed to achieve normality. For pollutant concentrations below the limit of quantification (LOQ), a LOQ × detection frequency value was assigned (Vanden Berghe et al., 2012). The respective LOQs are given in Table S1. Statistical analyses were conducted using SAS 9.3 software (SAS Institute Inc., Cary, USA). Linear mixed models were used to test differences in pollutant levels and profiles throughout the post-weaning fast periods. Animal ID was modelled as a random effect. In addition, the potential correlation between HO-PCBs and their precursors

Table 1
Concentrations of HO-PCB congeners and their potential PCB precursors, PCP and HCB in serum of weaned NES pups, expressed in pg/mL wet weight. The ratios of measured HO-PCBs and measured PCBs are also shown. Data represent the mean ± SEM of 22 sampled animals at weeks 1, 4 and 7 and 14 sampled animals at week 10.

	Period of fast			
	Week 1	Week 4	Week 7	Week 10
4-HO-CB107	135 ± 12 ^{a}	147 ± 13 ^{a}	184 ± 17 ^{b}	310 ± 24 ^{c}
4-HO-CB120	33 ± 2 ^{a}	35 ± 3 ^{a}	38 ± 3 ^{b}	56 ± 4 ^{c}
4-HO-CB146	44 ± 2 ^{a}	48 ± 2 ^{b}	54 ± 3 ^{c}	77 ± 3 ^{d}
4-HO-CB162	46 ± 4 ^{a}	44 ± 4 ^{a,b}	43 ± 4 ^{b}	47 ± 4 ^{a,b}
4-HO-CB163	4.3 ± 0.3 ^{a}	3.5 ± 0.4 ^{b}	4.8 ± 0.4 ^{a}	6.7 ± 0.4 ^{c}
4-HO-CB172	14 ± 1 ^{a}	14 ± 1 ^{a}	15 ± 1 ^{a}	17 ± 1 ^{b}
4-HO-CB187	6.9 ± 0.4 ^{a,b}	6.4 ± 0.5 ^{a}	7.7 ± 0.5 ^{b,c}	8.3 ± 0.4 ^{c}
∑ HO-PCBs	**282 ± 20^{a}**	**299 ± 19^{a}**	**346 ± 23^{b}**	**529 ± 31^{c}**
CB-105	109 ± 9 ^{a}	150 ± 14 ^{b}	178 ± 14 ^{c}	191 ± 19 ^{d}
CB-118	470 ± 23 ^{a}	634 ± 37 ^{b}	791 ± 42 ^{c}	901 ± 57 ^{d}
CB-138	588 ± 26 ^{a}	722 ± 33 ^{b}	931 ± 45 ^{c}	1067 ± 61 ^{d}
CB-146	71 ± 4 ^{a}	84 ± 4 ^{b}	123 ± 6 ^{c}	141 ± 11 ^{c}
CB-153	739 ± 31 ^{a}	860 ± 37 ^{b}	1156 ± 58 ^{c}	1312 ± 79 ^{d}
CB-170	36 ± 3 ^{a}	32 ± 2 ^{a}	50 ± 3 ^{b}	50 ± 4 ^{b}
CB-180	131 ± 11 ^{a}	124 ± 7 ^{a}	182 ± 12 ^{b}	191 ± 15 ^{b}
CB-183	37 ± 3 ^{a}	37 ± 4 ^{a}	49 ± 4 ^{b}	58 ± 4 ^{b}
CB-187	144 ± 12 ^{a}	132 ± 6 ^{a}	182 ± 11 ^{b}	207 ± 13 ^{c}
∑PCBs	**3723 ± 172^{a}**	**4533 ± 246^{b}**	**5058 ± 248^{c}**	**5851 ± 348^{d}**
∑HO-PCBs/∑PCBs	**0.068 ± 0.006^{a}**	**0.061 ± 0.005^{b}**	**0.064 ± 0.004^{a,b}**	**0.087 ± 0.006^{c}**
PCP	**103 ± 7^{a}**	**70 ± 9^{b}**	**146 ± 23^{a}**	**246 ± 41^{c}**
HCB	**168 ± 12^{a}**	**309 ± 14^{b}**	**253 ± 16^{c}**	**430 ± 29^{d}**

Values within a row followed by different letters are significantly different ($p \leq 0.05$).

were tested with a Pearson test. The level of statistical significance was set at p-values < 0.05 for all analyses.

3. Results

3.1. Levels and patterns of HO-PCBs

Among the twelve HO-PCBs that were targeted, four were under the LOQ in all samples (4′-HO-CB127, 3-HO-CB138, 4-HO-CB193 and 4-HO-CB208). Congener 4′-HO-CB130 was quantified in only a few samples (detection frequency=0% at week 1, 4 and 7 and 41% at week 10). Detection frequency was $> 50\%$ in two HO-penta-CBs (4-HO-CB107 and 120), three HO-hexa-CBs (4-HO-CB146, 162 and 163) and two HO-hepta-CBs (4-HO-CB172 and 187) (see Supplementary data).

ΣHO-PCB serum concentrations remained constant during the first 3 weeks of fast ($p=0.090$) and then, increased significantly until week 10 ($p < 0.001$) (Table 1). When considering individual congeners, all HO-PCBs significantly increased in serum between weeks 1 and 10 ($0.001 < p < 0.006$), except 4-HO-CB162 that remained constant ($p=0.492$) (Table 1). Moreover, the rise of bioaccumulation of penta-HO-CBs between weeks 1 and 10 (i.e. 4-HO-CB-107 and 4-HO-CB-120) seemed to be greater (mean factor of 2.0) than the one of hepta-HO-CBs (i.e. 4-HO-CB-172 and 4-HO-CB-187) (mean factor of 1.4) followed by the one of hexa-HO-CBs (i.e. 4-HO-CB-146, 4-HO-CB-162 and 4-HO-CB-163) (mean factor of 1.2).

Congener 4-HO-CB107 composed almost one half of ΣHO-PCBs (from $46.4 \pm 1.5\%$ at week 1 to $57.5 \pm 1.3\%$ at week 10). It was followed in a decreasing order by 4-HO-CB146 (from $16.7 \pm 1.1\%$ at week 1 to $15.1 \pm 0.6\%$ at week 10), 4-HO-CB162 (from $16.1 \pm 0.9\%$ at week 1 to $9.0 \pm 1.0\%$ at week 10), 4-HO-CB120 (from $10.9 \pm 0.2\%$ at week 1 to $10.5 \pm 0.3\%$ at week 10), 4-HO-CB172 (from $5.6 \pm 0.5\%$ at week 1 to $3.4 \pm 0.2\%$ at week 10), 4-HO-CB187 (from $2.7 \pm 0.2\%$ at week 1 to $1.7 \pm 0.2\%$ at week 10) and 4-HO-CB163 (from $1.6 \pm 0.1\%$ at week 1 to $1.3 \pm 0.1\%$ at week 10).

When HO-PCBs were grouped per class of chlorination degree, the main class was HO-penta-CBs followed by HO-hexa-CBs and HO-hepta-CBs (Fig. 2A).

3.2. Levels and patterns of PCBs

Among the thirty PCBs analysed in the serum, CB-172 and -199 were detected in only a few samples (see Supplementary data). Four congeners of PCBs (CB-174, -194, -206 and -209) were not analysed in any investigated sample (see Supplementary data).

ΣPCB concentrations in the serum increased significantly throughout the fasting period ($0.001 < p < 0.004$) (Table 1). Details concerning the levels and profiles of the thirty PCBs analysed in the serum are described elsewhere (Louis et al., 2014). Some PCBs detected in serum are considered as potential precursors of HO-PCBs (Table 2). The concentrations of all these precursors increased significantly from early to late fast ($0.001 < p < 0.017$) (Table 1).

When the results were expressed by homologue group in percentage of ΣPCBs, we found mainly hexa-CBs, followed by penta-CBs , tetra-CBs, hepta-CBs and tri-CBs for the first part of the fast and hexa-CBs, followed by penta-CBs, hepta-CBs, tetra-CBs and then, tri-CBs for the second part of the fast (Fig. 2B).

3.3. Correlations between HO-PCBs and PCBs

The ratios between the concentrations of ΣHO-PCBs and ΣPCBs decreased significantly between weeks 1 and 4 ($p=0.018$), remained constant between weeks 4 and 7 ($p=0.651$) and rose between weeks 7 and 10 ($p < 0.001$) (Table 1). There was a significant positive correlation between the concentrations of ΣHO-PCBs and ΣPCBs throughout the fast (Pearson correlation coefficient $r=0.539$, $p < 0.001$).

The correlations between individual HO-PCBs and their potential precursors of PCBs were also tested during the entire fasting period (Table 2). Fig. 3 illustrates an example of relationship between HO-PCBs (4-HO-CB146) and their precursors (CB-138, -146 and -153). Regarding HO-PCBs obtained by direct HO-

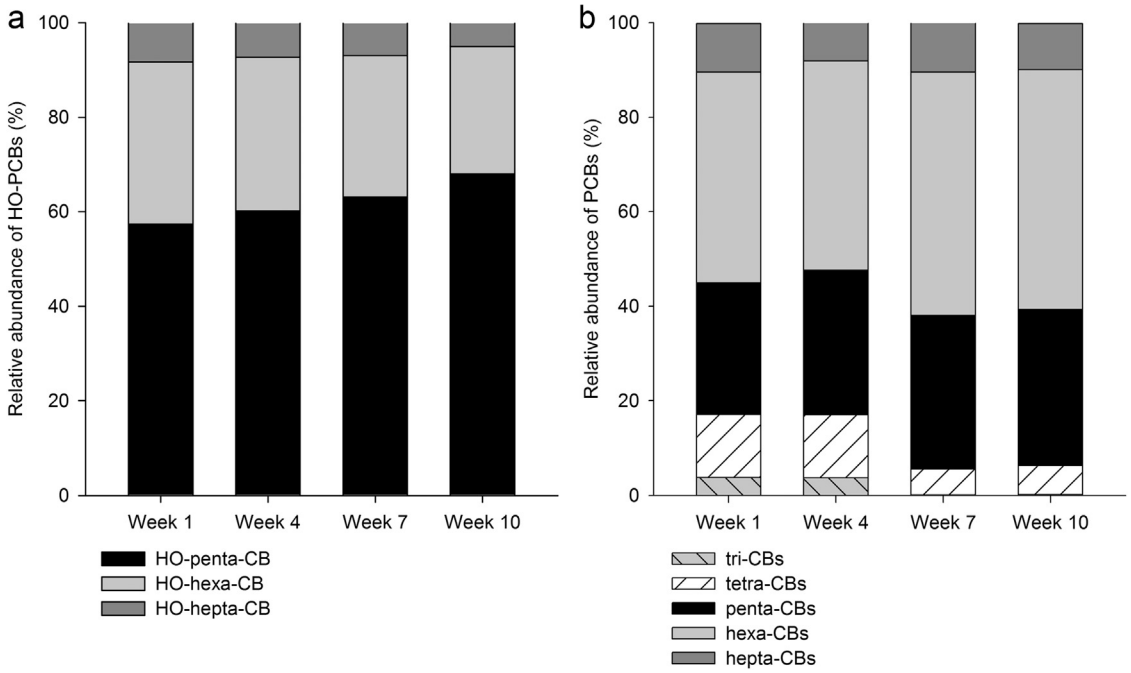

Fig. 2. Relative proportions of the different classes of HO-PCBs (a) and PCBs (b) in serum of NES pups during the post-weaning fast. Data were from 22 animals longitudinally sampled at weeks 1, 4 and 7. Fourteen of them were sampled at week 10.

Table 2
Pearson correlation coefficients (r) for individual HO-PCBs and their potential PCB precursors by direct HO-insertion (values in bold) and by the NIH-shift (values in italics) (pathways of biotransformation were compiled from Dirtu et al. (2010) and Weijs et al. (2009)). Data were from 22 sampled animals at weeks 1, 4 and 7 and 14 sampled animals at week 10. Values with an asterisk correspond to significant correlations ($p < 0.05$).

	PCB precursors	r_{total}	$r_{week\ 1}$	$r_{week\ 4}$	$r_{week\ 7}$	$r_{week\ 10}$
4-HO-CB107	CB-105	*0.217*	*−0.243*	*−0.170*	*0.132*	*0.182*
	CB-107 (N.A)	–				
	CB-118	*0.493**	*0.099*	*0.263*	*0.347*	*0.411*
4-HO-CB120	CB-118	*0.445**	*0.118*	*0.278*	*0.216*	*0.297*
	CB-120 (N.A)	–				
4-HO-CB146	CB-138	*0.709**	*0.542**	*0.368*	*0.538**	*0.538**
	CB-146	**0.652***	**0.459***	**0.186**	**0.542***	**0.433**
	CB-153	*0.691**	*0.538**	*0.433**	*0.477**	*0.424*
4-HO-CB162	CB-157 (N.A)	–				
	CB-162 (N.A)	–				
	CB-167 (N.A)	–				
4-HO-CB163	CB-158 (N.A)	–				
	CB-163 (N.A)	–				
4′-HO-CB172	CB-170	*0.246**	*0.385*	*−0.015*	*0.255*	*−0.072*
	CB-172 (N.D)	–				
	CB-180	*0.267**	*0.212*	*0.082*	*0.159*	*0.125*
4-HO-CB187	CB-183	*0.171*	*−0.253*	*0.140*	*0.359*	*−0.345*
	CB-187	**0.137**	**−0.323**	**0.219**	**0.159**	**−0.046**

N.A=not analysed; N.D=not detected in serum.

insertion, the concentrations of 4-HO-CB146 were positively correlated to the concentrations of CB-146 ($p < 0.001$), whereas the levels of 4-HO-CB187 were not correlated to the levels of CB-187 ($p=0.227$). Regarding the metabolism by NIH-shift, the levels of 4-HO-CB107 in the serum were positively correlated to the sum of CB-105 and CB-118 ($r=0.449$, $p < 0.001$). Considering the PCB precursors separately, 4-HO-CB107 was significantly correlated to CB-118 ($p < 0.001$), but not to CB-105 ($p=0.053$). The concentrations of 4-HO-CB120 were significantly correlated to the concentrations of CB-118 ($p < 0.001$). Similar observations were seen between 4-HO-CB146 and the sum of CB-138 and -153 ($r=0.710$, $p < 0.001$) as well as between 4-HO-CB146 and the precursors taken individually ($p < 0.001$) (Table 2 and Fig. 3). A significant correlation was noted between 4′-HO-CB172 and the sum of CB-170 and -180 ($r=0.268$, $p=0.016$) as well as between 4′-HO-CB172 and the precursors taken individually ($p=0.028$ for CB-170 and $p=0.017$ for CB-180). Finally, the concentration of 4-HO-CB187 was not correlated to CB-183 ($p=0.139$). The correlation coefficients constantly increased over time for 4-HO-CB107 and its PCB precursors. On the other hand, no trend could be highlighted for the other studied HO-PCB congeners and PCB precursors.

3.4. Concentrations of pentachlorophenol

The dynamics of PCP in serum differed somewhat from ΣHO-PCBs, with a significant decrease between weeks 1 and 4 ($p < 0.001$), followed by an increase during the rest of the fast ($p < 0.001$) (Table 1). PCP may be formed via HCB metabolism, but it can also directly bioaccumulate from the environment as it has been used as wood preservative until the 1980s. HCB was analysed in these same serum samples and results are described elsewhere (Louis et al., 2014). There was a good correlation between PCP and HCB levels ($r=0.334$; $p=0.002$).

4. Discussion

The presence of HO-PCBs in the serum of weaned NES pups may have several origins. They may come from the mother, via a placental transfer and/or the ingestion of low amounts present in the milk. Once absorbed by the pups, HO-PCBs tend to accumulate in blood, because of their high affinity for plasma proteins (Letcher et al., 2000; Vanden Berghe et al., 2012). They can also be stored in the liver, as already shown in harbour seals (Park et al., 2009). The presence of HO-PCBs in weaned NES pups may also result from an endogenous biotransformation of PCBs through the activity of CYP enzymes, which were detected in several phocid seal species (ringed, harbour, harp and grey seals) (Nyman et al., 2000; Ruus et al., 2002; Tilley et al., 2002; Wolkers et al., 1998, 2000). The concentrations of ΣHO-PCBs were more than one order of magnitude lower than the concentrations of ΣPCBs in weaned NES pups, leading to HO-PCB/PCB concentration ratios lower than 0.1. Several cetacean species, such as Pacific white-sided dolphin (*Lagenorhynchus obliquidens*) and beluga whale (*Delphinapterus leucas*), had HO-PCB/PCB concentration ratios lower than those measured in NES pups, whereas other marine mammals, including Bryde's whale (*Balaenoptera brydei*) and Minke whale (*Balaenoptera acutorostrata*), bottlenose dolphins (*Tursiops truncatus*), northern fur seals (*Callorhinus ursinus*) and harbour seals, showed HO-PCB/PCB concentration ratios similar to those found in weaned NES pups. Contrarily, the HO-PCB/PCB concentration ratios of killer whales (*Orcinus orca*), ringed seals (*Phoca hispida*) and grey seals (lactating females and suckling pups) were much greater than in NES pups. In the same way, NES pups do not seem to easily form HO-PCBs compared to polar bears (*Ursus maritimus*), a semi-marine species, as well as terrestrial mammals such as humans, raccoon dogs (*Nyctereutes procyonoides*), pet cats and pet dogs (Table 3). These differences might be due to interspecies variation of PCB biotransformation capacity. In addition, the induction of metabolising enzymes can be influenced by the hepatic levels of PCBs (Kunisue and Tanabe, 2009; Routti et al., 2008), which can vary between species, according to their PCB exposure.

The biotransformation of PCBs into HO-PCBs is a complex mechanism involving either a direct insertion of HO-group or a formation of arene oxide intermediate (Fig. 1). Most of the HO-PCB congeners were correlated to the different parent PCBs that were analysed. It may indicate that there is no preferential pathway of biotransformation in fasting NES pups. Nevertheless, further data are needed to confirm this hypothesis, since we detected only two PCB precursors (CB-146 and CB-187) of direct HO-insertion.

HO-penta-CBs composed more than half of the ΣHO-PCBs in NES pup serum. The preferential accumulation of this homologue group was also noted in the circulation of grey seals from Scotland (Vanden Berghe et al., 2012), harbour seals from the Atlantic and

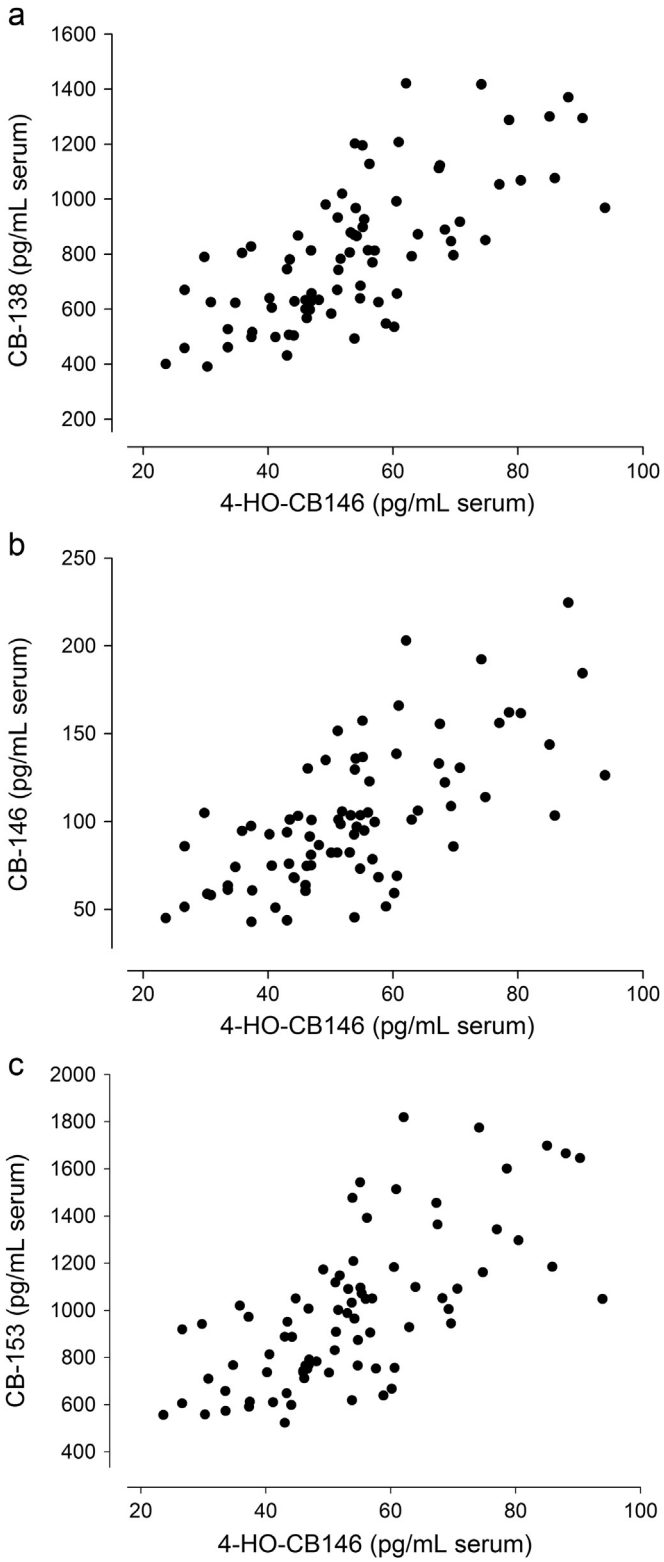

Fig. 3. Illustrative example of the relationship between HO-PCBs and their PCB precursors in serum: 4-HO-CB146 and CB-138 ($r=0.709$; $p<0.001$) (a), CB-146 ($r=0.652$; $p<0.001$) (b) and CB-153 ($r=0.691$; $p<0.001$) (c), expressed in pg/mL wet weight. Data represent the results of 22 sampled animals at weeks 1, 4 and 7 and 14 sampled animals at week 10.

the North Sea (Løken et al., 2008; Weijs et al., 2009) and different species of whales from the Japanese coast (melon-headed whale (*Peponocephala electra*), Stejneger's beaked whale (*Mesoplodon stejnegeris*), Pacific white-sided dolphin (*Lagenorhynchus obliquidens*), Blainville's beaked whale (*Mesoplodon densirostris*) and killer whale) (Nomiyama et al., 2010) as well as in the liver of beluga whales from Canada (McKinney et al., 2006). The domination of HO-penta-CBs is thus common to many marine species (Nomiyama et al., 2010). Such similarity across the world most probably implies that the biotransformation of PCBs in marine mammals is catalysed by the same CYP isozymes (Gabrielsen et al., 2011). However, this accumulation pattern of HO-penta-PCBs is not comparable with terrestrial mammals, such as dogs and raccoon dogs, which accumulate larger proportions of the higher chlorinated HO-PCBs, such as HO-hepta-CBs and HO-octa-CBs (Kunisue and Tanabe, 2009). The levels of HO-hexa-CBs were higher than HO-penta-CBs in human serum (Dirtu et al., 2010; Dirtu et al., 2013). Differences in the HO-PCB accumulation patterns observed between marine and terrestrial mammals might be caused by evolutionary differences in biotransformation enzymes, but also by differences in PCB exposure.

The levels of ΣHO-PCBs increased significantly in the serum of weaned NES pups from mid to late fast. Similarly, there was also an increase of HO-PCB/PCB concentration ratios at late fast. This increase in metabolites with time might result from a mobilisation of HO-PCBs from storage tissues, such as the liver, associated with the fast. There might also be an incomplete Phase II biotransformation of PCBs, resulting in an inefficient excretion of metabolites. Indeed, a previous study highlighted that *para* substituted HO-PCBs (like all analyzed HO-PCBs in this study) are resistant to the UDPGT conjugation in rat hepatic microsomes (Tampal et al., 2002), which, to some extent, could be at the origin of the rise of HO-PCB concentrations in NES blood during the fast. In addition, the *para*-HO substitution gives a similar structure to T_4, the natural substrate of TTR, to HO-PCBs (Lans et al., 1993; Letcher et al., 2000). The affinity of HO-PCBs for TTR may add to their retention and their increase in the blood. The absence of food intake for NES pups may influence the formation of HO-PCB metabolites by increasing the bioavailability of PCBs, among others through their (re)mobilisation from blubber (Debier et al., 2006; Louis et al., 2014), and by upregulating the CYP enzymes. For example, the concentrations of HO-PCBs were higher in fasting ringed seals than in non-fasting animals (Routti et al., 2010). The presence of PCBs has been shown to promote the expression of CYP1A in the liver of fasting fish (Vijayan et al., 2006). Fasting itself seems to increase the expression of genes encoding for the biotransformation enzymes in fish and rodents (Cheesman and Reilly, 1998; Ding et al., 2006). Likewise, the biotransformation capacity and the HO-PCB metabolites increase during food deprivation episodes in herring gull chicks (Routti et al., 2013). Consequently, we may suggest a positive impact of the post-weaning fast of NES on the formation of HO-PCBs and their accumulation in blood.

Eventually, the increase of HO-PCB/PCB concentration ratios of weaned NES pups from mid fast might translate an increase of metabolic capacity with age of NES. Indeed, it has been previously shown that CYP activities increase with age in humans (Milsap and Jusko, 1994) and that the age and the levels of HO-PCBs in hooded seal pups are positively correlated (Gabrielsen et al., 2011). Based on HO-PCB/PCB concentration ratios (Table 3), it seems that we cannot extrapolate this observation to all mammals since grey seal suckling pups had values of ratio similar to grey seal lactating females. Nevertheless, the HO-PCB/PCB concentration ratios of grey seal suckling pups can be influenced by those of the females since PCBs and, to some extent, HO-PCBs, can be transferred from mother to pup through the milk (Vanden Berghe et al., 2012). It could be interesting to study the HO-PCB/PCB concentration ratios in fasting NES at different age stages (pups, juveniles and adults), in comparable physiological states, in order to confirm the link between age and biotransformation capacity.

Table 3
Mean HO-PCB/PCB concentration ratios in different species of mammals.

Species	Age	HO-PCB/PCB ratios	References
Pacific white-sided dolphin		0.0015	Nomiyama et al. (2010)
Beluga whale		0.0045	
Bryde's whale		0.013	
Minke whale		0.069	
Bottlenose dolphin	Juveniles and adults	0.016	Houde et al. (2006)
Northern elephant seal	**Weaned pups**	**0.061–0.087**	**Present study**
Northern fur seal		0.065	Kunisue and Tanabe (2009)
Harbour seal	Juveniles and adults	0.086	Weijs et al. (2009)
Human	Adults	0.11	Sandau et al. (2000a)
Killer whale	Adults	0.170	Bennett et al. (2009)
Ringed seal	Adults	0.240	Routti et al. (2008)
Human	Adults	0.33	Sandau et al. (2000a)
Human	Adults	0.37	Kunisue and Tanabe (2009)
Grey seal	Suckling pups	0.523–0.742	Vanden Berghe et al. (2012)
Grey seal	Lactating females	0.515–1.004	
Polar bear	Juveniles and adults	1.97	Sandala et al. (2004)
Raccoon dog		4.3	Kunisue and Tanabe (2009)
Cat		5.3	
Dog		29	

It seems important to note that the increase of HO-PCBs differed between the congeners. The rise of concentration was indeed more important for the less chlorinated congeners compared to the more chlorinated ones. Louis et al. (2014) noted a more pronounced rise of penta-CBs, followed by hexa-CBs and then, hepta-CBs in the serum of weaned NES pups. It thus seems reasonable to think that the higher disponibility of less chlorinated PCBs in serum promoted their biotransformation by peripheral tissues such as the liver. Moreover, Safe (1989) reported that PCBs with more than five chlorine atoms are more resistant to biotransformation than the less chlorinated ones, leading to a higher production of less chlorinated HO-PCB congeners.

The concentrations of PCP in the serum of weaned NES pups were in the same order of magnitude as those found in the blood of harbour seals from the North Sea (Dupont et al., 2013) and northern fur seals from Japan (Kunisue and Tanabe, 2009), in the plasma of ringed seals from Canada (Sandau et al., 2000b), polar bears and ringed seals from Canada (Sandau et al., 2000b) and one captive killer whale from Canada (Bennett et al., 2009). The serum of NES weaned pups was 2–4 fold less contaminated by PCP than the plasma of ringed seals from Svalbard and the Baltic Sea (Routti et al., 2009) and one order of magnitude less contaminated than the plasma of bowhead whales (*Balaena mysticetus*) from Alaska (Hoekstra et al., 2003). PCP may have two origins in the NES serum: it can either result from maternal transfer or be formed by the pups themselves via HCB metabolism. As a good correlation was noted between PCP and HCB, we can expect that weaned NES pups are able to biotransform HCB, the parent molecule, in PCP (Renner, 1988).

The results of this study indicate that NES pups were contaminated by HO-PCBs and their concentrations in serum increased throughout the post-weaning fast. As a consequence, the increase of HO-PCB levels might have adverse effects on health of NES in full growth. It appears from our data that NES pups are able to biotransform the PCBs into HO-PCBs although HO-PCBs might also originate from placental transfer and/or ingestion of milk. In agreement with the literature, the capacity of PCB biotransformation seems to be influenced by several factors (age, species, physiological status of the animal, PCB exposure, etc.).

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Appendix A. Supplementary Information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2014.08.040.

References

Bennett, E.R., et al., 2009. Chlorinated and brominated organic contaminants and metabolites in the plasma and diet of a captive killer whale (Orcinus orca). Mar. Pollut. Bull. 58, 1078–1083.
Bytingsvik, J., et al., 2012. PCBs and OH-PCBs in polar bear mother–cub pairs: a comparative study based on plasma levels in 1998 and 2008. Sci. Total Environ. 417, 117–128.
Cheesman, M.J., Reilly, P.E.B., 1998. Differential inducibility of specific mRNA corresponding to five CYP3A isoforms in female rat liver by RU486 and food deprivation: Comparison with protein abundance and enzymic activities. Biochem. Pharmacol. 56, 473–481.
Connor, K., et al., 1997. Hydroxylated polychlorinated biphenyls (PCBs) as estrogens and antiestrogens: structure-activity relationships. Toxicol. Appl. Pharmacol. 145, 111–123.
Covaci, A., et al., 2002. Distribution of organobrominated and organochlorinated contaminants in Belgian human adipose tissue. Environ. Res. 88, 210–218.
Daly, J., et al., 1972. Arene oxides and the NIH shift: the metabolism, toxicity and carcinogenicity of aromatic compounds. Experientia 28, 1129–1149.
Debier, C., et al., 2006. Mobilization of PCBs from blubber to blood in northern elephant seals (Mirounga angustirostris) during the post-weaning fast. Aquatic Toxicol. 80, 149–157.
Debier, C., et al., 2012. Differential changes of fat-soluble vitamins and pollutants during lactation in northern elephant seal mother–pup pairs. Comp. Biochem. Physiol. Part A 162, 323–330.
Ding, X., et al., 2006. Regulation of constitutive androstane receptor and its target genes by fasting, cAMP, hepatocyte nuclear factor α, and the coactivator peroxisome proliferator-activated receptor γ coactivator-1α. J. Biol. Chem. 281, 26540–26551.
Dirtu, A.C., et al., 2013. Dynamics of organohalogenated contaminants in human serum from obese individuals during one year of weight loss treatment. Environ. Sci. Technol. 47, 12441–12449.

Dirtu, A.C., et al., 2010. Distribution of PCBs, their hydroxylated metabolites, and other phenolic contaminants in human serum from two European countries. Environ. Sci. Technol. 44, 2876–2883.
Dupont, A., et al., 2013. Relationships between in vitro lymphoproliferative responses and levels of contaminants in blood of free-ranging adult harbour seals (*Phoca vitulina*) from the North Sea. Aquat. Toxicol. 142–143, 210–220.
Gabrielsen, K.M., et al., 2011. Levels and patterns of hydroxylated polychlorinated biphenyls (OH-PCBs) and their associations with thyroid hormones in hooded seal (Cystophora cristata) mother-pup pairs. Aquat. Toxicol. 105, 482–491.
Hoekstra, P.F., et al., 2003. Hydroxylated and methylsulfone-containing metabolites of polychlorinated biphenyls in the plasma and blubber of bowhead whales (Balaena mysticetus). Environ. Toxicol. Chem. 22, 2650–2658.
Houde, M., et al., 2006. Polychlorinated biphenyls and hydroxylated polychlorinated biphenyls in plasma of bottlenose dolphins (Tursiops truncatus) from the Western Atlantic and the Gulf of Mexico. Environ. Sci. Technol. 40, 5860–5866.
Kunisue, T., Tanabe, S., 2009. Hydroxylated polychlorinated biphenyls (OH-PCBs) in the blood of mammals and birds from Japan: lower chlorinated OH-PCBs and profiles. Chemosphere 74, 950–961.
La Merrill, M., et al., 2013. Toxicological function of adipose tissue: focus on persistent organic pollutants. Environ. Health Perspect. 121, 162–169.
Lans, M.C., et al., 1993. Structure-dependent, competitive interaction of hydroxy-polychlorobiphenyls, -dibenzo-p-dioxins and -dibenzofurans with human transthyretin. Chemico-Biol. Interact. 88, 7–21.
Larsson, C., et al., 2004. Enantiomeric specificity of methylsulfonyl-PCBs and distribution of bis (4-chlorophenyl) sulfone, PCB, and DDE methyl sulfones in grey seal tissues. Environ. Sci. Technol. 38, 4950–4955.
Letcher, R., et al., 2000. In: Hutzinger, O., Paasivirta, J. (Eds.), Methyl sulfone and hydroxylated metabolites of polychlorinated biphenyls. Springer, Berlin/Heidelberg, pp. 315–359.
Letcher, R.J., et al., 2010. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. Sci. Total Environ. 408, 2995–3043.
Løken, K.B., et al., 2008. How important are the hydroxylated PCB metabolites in harbour seals (*Phoca vitulina*)? Organohalogen Compd. 70, 25–28.
Louis, C., et al., 2014. Mobilisation of lipophilic pollutants from blubber in northern elephant seal pups (*Mirounga angustirostris*) during the post-weaning fast. Environ. Res. 132, 438–448.
Maervoet, J., et al., 2004. A reassessment of the nomenclature of polychlorinated biphenyl (PCB) metabolites. Environ. Health Perspect. 112, 291–294.
McKinney, M.A., et al., 2006. Organohalogen contaminants and metabolites in beluga whale (*Delphinapterus leucas*) liver from two Canadian populations. Environ. Toxicol. Chem. 25, 1246–1257.
Meerts, I.A., et al., 2004. Developmental exposure to 4-hydroxy-2, 3, 3′, 4′, 5-pentachlorobiphenyl (4-OH-CB107): long-term effects on brain development, behavior, and brain stem auditory evoked potentials in rats. Toxicol. Sci. 82, 207–218.
Milsap, R.L., Jusko, W.J., 1994. Pharmacokinetics in the infant. Environ. Health Perspect. 102 (Suppl 11), 107–110.
Narasimhan, T., et al., 1991. Effects of hydroxylated polychlorinated biphenyls on mouse liver mitochondrial oxidative phosphorylation. J. Biochem. Toxicol. 6, 229–236.
Nomiyama, K., et al., 2010. Polychlorinated biphenyls and their hydroxylated metabolites (OH-PCBs) in the blood of toothed and baleen whales stranded along Japanese coastal waters. Environ. Sci. Technol. 44, 3732–3738.
Noren, D.P., et al., 2003. Energy reserve utilization in northern elephant seal (Mirounga angustirostris) pups during the postweaning fast: size does matter. J. Comparative Physiol. B 173, 443–454.
Nyman, M., et al., 2000. Expression and inducibility of members in the cytochrome P4501 (CYP1) family in ringed and grey seals from polluted and less polluted waters. Environ. Toxicol. Pharmacol. 8, 217–225.
Nyman, M., et al., 2001. Characterization of xenobiotic-metabolizing cytochrome P450 (CYP) forms in ringed and grey seals from the Baltic Sea and reference sites. Comparative Biochem. Physiol. Part C 128, 99–112.
Park, J.-S., et al., 2009. Polychlorinated biphenyls (PCBs) and their hydroxylated metabolites (OH-PCBs) in livers of harbor seals (Phoca vitulina) from San Francisco Bay, California and Gulf of Maine. Mar. Environ. Res. 67, 129–135.
Renner, G., 1988. Hexachlorobenzene and its metabolism. Toxicol. Environ. Chem. 18, 51–78.
Routti, H., et al., 2013. Effect of reduced food intake on toxicokinetics of halogenated organic contaminants in herring gull (*Larus argentatus*) chicks. Environ. Toxicol. Chem. 32, 156–164.
Routti, H., et al., 2010. Hormone, vitamin and contaminant status during the moulting/fasting period in ringed seals (Pusa [*Phoca*] *hispida*) from Svalbard. Comparative Biochem. Physiol. Part A 155, 70–76.
Routti, H., et al., 2008. Biotransformation of PCBs in rxenobiotic-metabolizing enzyme activities in ringed seselation to Phase I and II Xenobiotic-metabolizing enzyme activities in Ringed Seals (*Phoca hispida*) from Svalbard and the Baltic Sea. Environ. Sci. Technol. 42, 8952–8958.
Routti, H., et al., 2009. Concentrations, patterns and metabolites of organochlorine pesticides in relation to xenobiotic phase I and II enzyme activities in ringed seals (*Phoca hispida*) from Svalbard and the Baltic Sea. Environ. Pollut. 157, 2428–2434.
Ruus, A., et al., 2002. Factors influencing activities of biotransformation enzymes, concentrations and compositional patterns of organochlorine contaminants in members of a marine food web. Aquat. Toxicol. 61, 73–87.
Safe, S., 1989. Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products131–159.
Sandala, G.M., et al., 2004. Hydroxylated and methyl sulfone PCB metabolites in adipose and whole blood of polar bear (*Ursus maritimus*) from East Greenland. Sci. Total Environ. 331, 125–141.
Sandau, C.D., et al., 2000a. Analysis of hydroxylated metabolites of PCBs (OH-PCBs) and other chlorinated phenolic compounds in whole blood from Canadian inuit. Environ. Health Perspect. 108, 611–616.
Sandau, C.D., et al., 2002. Pentachlorophenol and hydroxylated polychlorinated biphenyl metabolites in umbilical cord plasma of neonates from coastal populations in Quebec. Environ. Health Perspect. 110, 411–417.
Sandau, C.D., et al., 2000b. Identification of 4-hydroxyheptachlorostyrene in polar bear plasma and its binding affinity to transthyretin: a metabolite of octachlorostyrene? Environ. Sci. Technol. 34, 3871–3877.
Schuur, A.G., et al., 1998. In vitro inhibition of thyroid hormone sulfation by hydroxylated metabolites of halogenated aromatic hydrocarbons. Chem. Res. Toxicol. 11, 1075–1081.
Tampal, N., et al., 2002. Glucuronidation of hydroxylated polychlorinated biphenyls (PCBs). Chem. Res. Toxicol. 15, 1259–1266.
Teramitsu, I., et al., 2000. Identification of novel cytochrome P450 1A genes from five marine mammal species. Aquat. Toxicol. 51, 145–153.
Tilley, R.E., et al., 2002. Isolation of two cytochrome P450 cDNAs, CYP1A1 and CYP1A2, from harp seal (*Phoca groenlandica*) and grey seal (*Halichoerus grypus*). Comparative Biochem. Physiol. Part C 132, 181–191.
Uhl, S., et al., 1986. Pharmacokinetics of pentachlorophenol in man. Arch. Toxicol. 58, 182–186.
Van den Berg, K., et al., 1991. Interactions of halogenated industrial chemicals with transthyretin and effects on thyroid hormone levels in vivo. Arch. Toxicol. 65, 15–19.
Van der Plas, S.A., et al., 2001. Effects of subchronic exposure to complex mixtures of dioxin-like and non-dioxin-like polyhalogenated aromatic compounds on thyroid hormone and vitamin A levels in female Sprague-Dawley rats. Toxicol. Sci. 59, 92–100.
Vanden Berghe, M., et al., 2013. Effects of polychlorobiphenyls, polybromodiphenylethers, organochlorine pesticides and their metabolites on vitamin A status in lactating grey seals. Environ. Res. 120, 18–26.
Vanden Berghe, M., et al., 2012. Selective transfer of persistent organic pollutants and their metabolites in grey seals during lactation. Environ. Int. 46, 6–15.
Vijayan, M.M., et al., 2006. Fasting augments PCB impact on liver metabolism in anadromous Arctic char. Toxicol. Sci. 91, 431–439.
Villanger, G.D., et al., 2013. Effects of complex organohalogen contaminant mixtures on thyroid homeostasis in hooded seal (*Cystophora cristata*) mother–pup pairs. Chemosphere 92, 828–842.
Weijs, L., et al., 2009. Concentrations of chlorinated and brominated contaminants and their metabolites in serum of harbour seals and harbour porpoises. Env. Int. 35, 842–850.
Wolkers, H., et al., 2000. Chlorinated pesticide concentrations, with an emphasis on polychlorinated camphenes (toxaphenes), in relation to cytochrome P450 enzyme activities in harp seals (*Phoca groenlandica*) from the Barents Sea. Environ. Toxicol. Chem. 19, 1632–1637.
Wolkers, J., et al., 1998. Phase I and phase II enzyme activities in ringed seals (Phoca hispida): characterization of hepatic cytochrome P450 by activity patterns, inhibition studies, mRNA analyses, and western blotting. Aquat. Toxicol. 44, 103–115.
Zamek-Gliszczynski, M.J., et al., 2006. Integration of hepatic drug transporters and phase II metabolizing enzymes: mechanisms of hepatic excretion of sulfate, glucuronide, and glutathione metabolites. Eur. J. Pharm. Sci. 27, 447–486.